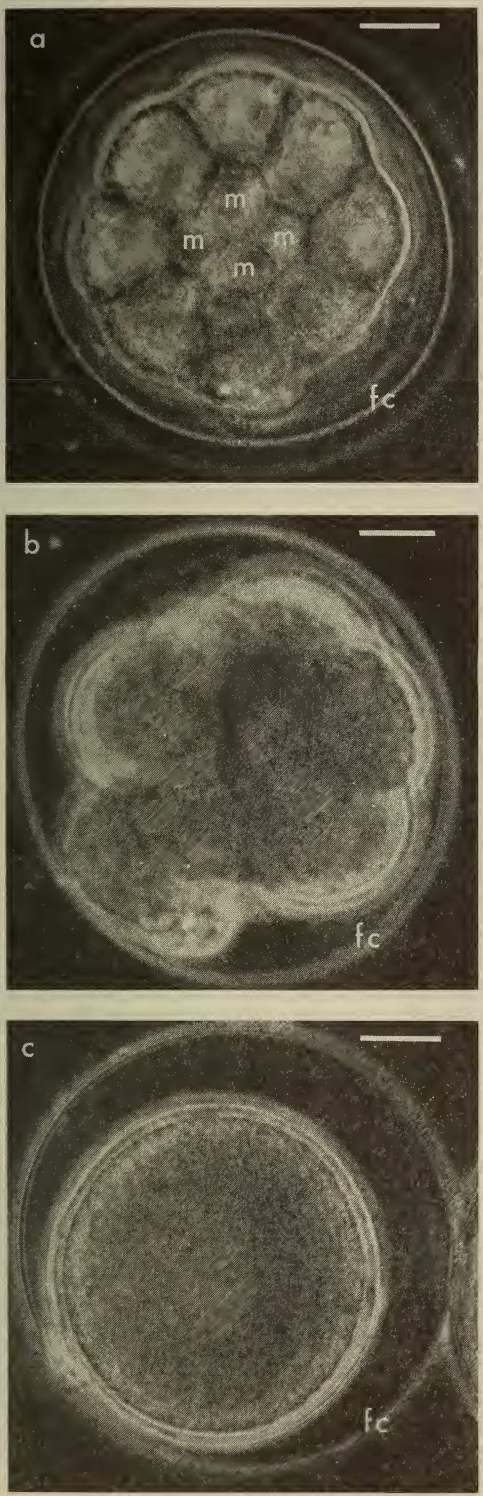

nent of thymidine uptake in activated eggs.

Effects of NBMPR on Early Embryonic Development

Figure 6a shows a sea urchin embryo cultured in ASW 6 hr after fertilization. Development to the sixteen-cell stage and appearance of a quartet of micromeres has proceeded normally. Figures 6b and 6c show embryos cultured for 6 hr in ASW containing 50 μM and 400 μM NBMPR, respectively. In the presence of 50 μM NBMPR, cell division proceeds but the normal cleavage planes are disrupted and a definitive quartet of micromeres fails to appear at the scheduled time. In 400 μM NBMPR, cleavage is totally inhibited. These results show that along with its inhibitory effects on thymidine uptake, NBMPR exerts inhibitory effects on cleavage and early embryonic development.

Discussion

In the present study, a drug known to inhibit nucleoside uptake in many types of vertebrate cells has been tested on fertilized sea urchin eggs. Nitrobenzylthioinosine (NBMPR) has been shown to bind to high affinity binding sites on the plasma membranes of human erythrocytes.[26,27] These sites are present at $1.0–1.5 \times 10^4$/cell, bind NBMPR with an apparent K_d of 1 nM, and, since inhibition of uridine uptake is proportional to the number of sites occupied by NBMPR, are presumed to play an important role in the nucleoside uptake mechanism.[27] Although nucleosides can compete with NBMPR for bind-

Fig. 6. Phase-Contrast micrographs of fertilized sea urchin eggs 6 hr post-insemination in ASW [a], ASW containing 50 μM NBMPR [b], and ASW containing 400 μM NBMPR [c]. fc = fertilization coat, m = micromeres, bars = 20 μm.

ing sites, Cass and Paterson have provided a cogent study which suggests that NBMPR binding sites are different from nucleoside uptake sites.[28]

Human erythrocytes are highly differentiated, anucleate cells and, as such, have lost their abilities to replicate DNA and synthesize RNA. The ability to metabolize transported nucleosides to the mono-, di-, and triphosphorylated nucleotides is also lost. On the other hand, all nucleated, dividing cells, including human cancer (HeLa) cells[29] and fertilized sea urchin eggs,[6–8,30] rapidly metabolize transported nucleosides. In HeLa cells, it has been shown that NBMPR is a potent inhibitor of nucleoside uptake,[24,29] but that thymidine and uridine kinase activities in cell extracts are unaffected by NBMPR concentrations well in excess of those which block nucleoside transport.[29] Apparently, NBMPR does not inhibit nucleoside transport in HeLa cells by inhibiting a metabolic coupling of transport with nucleoside phosphorylation. Rather, transport is blocked specifically.

Our results show that NBMPR also inhibits nucleoside uptake in fertilized sea urchin eggs, but to a lesser extent than that shown in other vertebrate cells. For example, while 5 μM NBMPR is sufficient to completely inhibit nucleoside uptake in HeLa cells, 400 μM NBMPR is required to achieve an 80% inhibition of thymidine uptake in fertilized sea urchin eggs. Additionally, the inhibition curve shown in Figure 2 indicates that a significant portion (ca., 20%) of thymidine uptake remains insensitive to NBMPR. This type of inhibition is apparently not limited to the early cleavage stages of development reported here, because virtually identical inhibition curves have been generated for 48 hr (late gastrula) embryos (Eagan and Nishioka, unpublished results).

Our measurements indicate that [^{3}H]-NBMPR binds to the fertilized egg surface, but that this binding is not Na^+-dependent. Since thymidine uptake has been shown to be very strictly Na^+-dependent, our results can be interpreted as further evidence in support of the idea that NBMPR binding sites are different from nucleoside uptake sites. Both [^{3}H]-NBMPR binding and [^{3}H]-thymidine uptake, on the other hand, are shown to be partially Ca^{2+}-dependent in parthenogenetically activated eggs. Binding is reduced 50% and uptake is reduced 20% in $0Ca^{2+}$-SW. This difference can also be interpreted as evidence for separate NBMPR binding sites and nucleoside uptake sites. More importantly, Ca^{2+} is known to be involved in many receptor-mediated cellular processes and our results indicate that both NBMPR binding and thymidine uptake in activated sea urchin eggs may now be added to this list.

The most equivocal aspects of this study are (1) the extremely high concentrations of NBMPR required to inhibit thymidine uptake and (2) the adverse effects it exerts on early development. The first aspect questions the specificity of NBMPR in inhibiting nucleoside uptake. At the high concentrations used, does NBMPR inhibit all uptake? We have measured the effects of 100 μM NBMPR on the uptake of the amino acids leucine, glycine, and lysine and have observed 20%, 30%, and 35% inhibitions, respectively (unpublished results), so there is in fact some nonspecific inhibition of uptake, but not enough to conclude that NBMPR is a completely nonspecific inhibitor. The second aspect questions whether NBMPR inhibition of thymidine uptake is a cause or an effect of the inhibition of cleavage. What is needed to settle this question are determinations of the effects of NBMPR on other post-fertilization metabolic processes. One such process for which the necessary techniques have just become available and which should also provide valuable information about the mechanism of nucleoside uptake, is the phosphorylation of transported nucleosides.[30] It could be that in fertilized sea urchin eggs, unlike HeLa cells, nucleoside uptake is dependent on metabolic coupling with phosphorylation. Experiments are

planned to determine the effects of NBMPR on the *in vivo* phosphorylation of transported nucleosides and the *in vitro* activities of the nucleoside kinases in sea urchin egg extracts.

Acknowledgments

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SURVIVAL OF *MORONE SAXATILIS* IN LOW pH OLIGOHALINE WATERS

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ABSTRACT

The hypothesis that declines in striped bass (*Morone saxatilis*) stocks are related to acid deposition in freshwater areas of the Chesapeake Bay estuary has become widespread. Attention has focused above the freshwater/saltwater interface due to the belief that low salinity would buffer waters from pH fluctuations.

Continuous water quality monitoring of Seneca Creek waters in the Upper Chesapeake Bay revealed sustained low pH during 1985, a dry year. Low pH levels were recorded despite increasing salinity. In April, salinities of ≤3 ppt were found with pH >7, but by May pH was <6 while salinities increased to 4 ppt. Low pH (<6) persisted until late August despite salinity increasing to 6 ppt. Water quality surveys of surrounding embayments revealed isolated pockets of low pH (<6) in Seneca Creek, Middle River and Gunpowder River. These findings demonstrate that acidic conditions are possible at low salinities and may be a common occurrence in poorly buffered oligohaline estuaries.

Low pH did not affect the survival of striped bass cultured in the Crane Aquaculture Facility during 1985. No difference was seen in survival between larvae held in buffered (pH ≥ 6.2) or ambient (pH as low as 5.3) water. Larvae were exposed to pH <6 at an age of 19 days with no apparent increase in mortality.

Eight facilities, from five states, contributed striped bass to the Chesapeake Bay Striped Bass Binary Coded Wire Tagging Project; a cooperative program of the U.S.F.W.S. and Maryland Department of Natural Resources. The Crane Aquaculture Facility contributed 21.8% of the numbers and 49.8% of the biomass, demonstrating successful production during 1985. This indicates acid deposition alone is not the major factor in striped bass declines in oligohaline areas of the Chesapeake Bay.

Introduction

In recent years the concern over the degradation of biological communities in estuaries via acid deposition has received attention in both scientific and popular literature. Attention has focused on possible pH shifts occurring near the freshwater/saltwater interface. Investigators appear to have assumed the buffering capacity of oligohaline water would not permit sustained low pH and thus few pH studies have been conducted below the

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interface. Data from organically rich blackwater rivers of the Southeastern Coastal Plain suggest this assumption is not valid for all estuaries[1].

The toxic effects of acid deposition in estuaries are thought to be caused by low pH pulses. These pulses are hypothesized to have been uncommon and organisms are thus susceptible to these events. Low pH has been suggested as one possible cause for declines of striped bass (*Morone saxatilis*) stocks in the Chesapeake Bay. Larval and juvenile fish are particularly susceptible to the pulses[2,3]. Reports in scientific[3] and popular[4] literature suggested toxic effects of acidification and aluminum on striped bass and other estuarine organisms. Most studies of pH effects on striped bass have been conducted in freshwater[2,5,6] since most culture facilities are situated in freshwater environs. It has been reported that larval striped bass survival increases with low salinity[7,8]. However, studies have not been conducted to determine effects of increased salinity on the toxicity of low pH.

The Crane Aquaculture Facility uses ambient oligohaline waters to culture striped bass. During 1985 sustained low pH occurred in the nearshore waters surrounding the Crane Facility. The objective of this publication is to describe this occurrence and its impact on striped bass cultured at the facility during 1985.

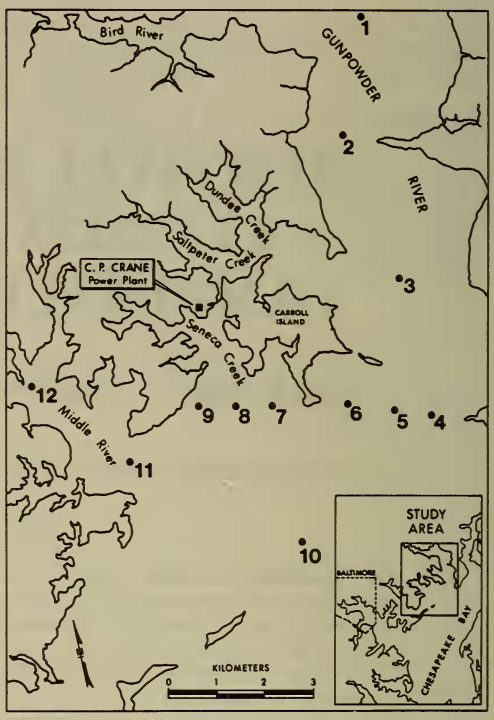

Fig. 1. Location of the Crane Aquaculture Facility at the Crane Power Plant on Seneca Creek and the stations sampled on water quality surveys.

Methods

The Crane Aquaculture Facility is located on Seneca Creek in the Upper Chesapeake Bay (Fig. 1). This facility has been operating since the spring of 1983 and utilizes a once-through system to deliver a minimum of 1250 gpm of either ambient water from Seneca Creek or warmed discharge water from a power plant for intensive culture of striped bass. Intake water is continuously monitored for temperature, conductivity, pH and dissolved oxygen. During 1985, ambient water was used for the period covered by this report after discharge canal pumps were turned off on 29 April.

Continuous water quality monitoring was conducted using Rexnord Model 3750/51 pH transmitters with Model 375 sensors, Leeds and Northrup Model 7073-17 Industrial conductivity monitors and Rexnord Model 3060 dissolved oxygen meters. Daily pH, dissolved oxygen, temperature and salinity samples were also taken. Water quality surveys were conducted on nearby embayments of the Chesapeake Bay after low pH was observed. The daily and survey water quality readings were taken using glass or plastic sampling devices, a YSI Model 51B Oxygen meter, a A0 hand refractometer, and a Fisher Model 800 Accumet pH meter.

Selected holding tanks for larvae were buffered to maintain pH levels above ambient waters. Reservoirs of bicarbonate

solution were used to maintain a controlled drip of buffer, via pumps, to mix with incoming ambient waters in the holding tanks. Other holding tanks received unbuffered ambient waters for comparison of larval survival.

Results

The pH of ambient waters flowing through Crane Aquaculture Facility decreased while salinities increased (Fig. 2). From April to May pH levels were ≥7 while salinities were ≤3 ppt. By the end of May the pH had dropped below 6 while salinity increased to 4 ppt. The pH remained consistently below 6 while salinity increased to 6 ppt by late August, after which pH levels started to rise.

To document the occurrence of low pH in the embayments near the Crane Facility water quality surveys were conducted at selected stations (Fig. 1). The first survey, 14 August, found pH levels below 6

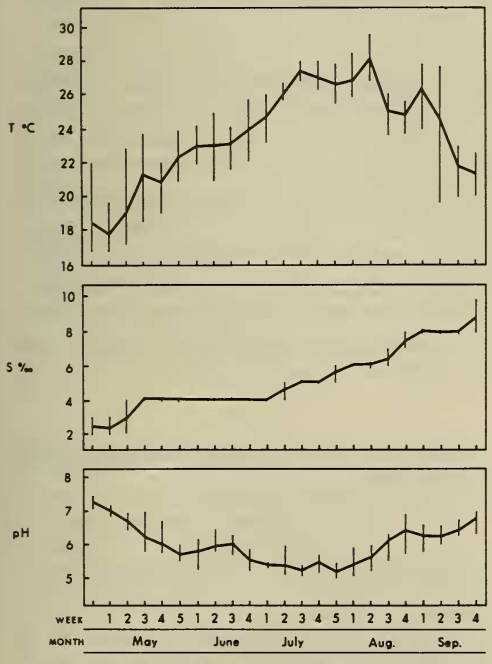

Fig. 2. Weekly averages and ranges for pH, salinity and temperature of intake waters of the Crane Aquaculture Facility for April–September 1985.

Table 1.—Results of water quality samples taken in a survey on 14 August 1985. Station locations are shown on Fig. 1.

Station	Depth (ft)	pH	Temp. (°C)	Salinity (%)
3	0	5.9	29.9	6.0
	2	5.9	28.3	6.0
4	0	6.8	29.4	6.0
	2	6.8	28.1	6.0
5	0	6.7	29.2	6.0
	2	6.7	28.0	6.0
6	0	6.8	29.1	6.0
	2	6.7	28.8	6.0
7	0	6.0	29.1	6.0
	2	5.9	28.0	6.0
8	0	5.9	29.1	6.0
	2	5.6	28.9	6.0
9	0	5.9	29.0	6.0
	2	5.9	28.8	6.0
10	0	7.8	28.9	7.0
	2	7.8	28.0	7.0
11	0	5.8	29.0	6.0
	2	5.7	28.8	6.0

Table 2.—Results of water quality samples taken in a survey on 19 August 1985. Station locations are shown on Fig. 1.

Station	Depth (ft)	pH	Temp. (°C)	Salinity (%)
1	0	6.8	27.1	4.5
	6	6.8	25.0	4.5
2	0	6.8	26.7	5.0
	6	5.9	25.0	5.0
3	0	5.6	26.0	6.0
	6	6.5	25.0	6.0
4	0	7.4	26.0	7.0
	6	7.4	24.8	7.0
5	0	7.2	25.7	7.0
	6	7.3	25.3	7.0
6	0	6.7	25.7	7.0
	6	7.0	25.2	7.5
7	0	6.2	25.1	6.0
	6	6.5	24.7	6.5
8	0	6.6	25.0	6.5
	6	6.7	25.0	6.5
9	0	6.5	25.3	7.0
	6	6.5	24.9	7.0
10	0	7.3	26.0	7.0
	6	7.3	25.8	8.0
11	0	5.3	25.3	6.0
	6	5.5	25.1	7.0